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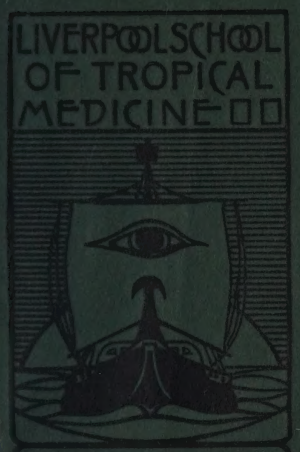

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The Origin and Development of Gametes (Crescents) in Malignant Tertian Malaria: Some Observations on Flagellation, Etc.

DAVID THOMSON

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THE ORIGIN AND DEVELOPMENT OF GAMETES (CRESCENTS) IN MALIGNANT TERTIAN MALARIA : SOME OBSERVATIONS ON FLAGELLATION, ETC.

BY

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(*Being Part II of the Report on the Malarial Expedition to Panama*, 1912)

(*Received for publication* 21 *January*, 1914)

PLATE V

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I. INTRODUCTION

The part of this research dealing with the study of crescents in autopsy smears of the inner organs was done in conjunction with Dr. W. M. James, Chief Assistant Physician at the Ancon Hospital, Panama, and to him is due the credit of tracing the various stages of the crescent from its earliest beginnings to the adult form. The observations on the crescents in the peripheral blood were made in the Royal Southern Hospital, Liverpool, and full details have already been published (Thomson, 1911). Dr. James continued the work by studying the development of crescents in the inner organs, and, together, we made further observations on this subject in Panama during October, November and December, 1912. The stained specimens of moist chamber crescents (figs. 31-41) were prepared by James.

II. DEDUCTIONS MADE FROM OBSERVATIONS ON CRESCENTS IN THE PERIPHERAL BLOOD

(*a*) *Each crescent develops from a single ordinary asexual spore of P. falciparum.* Mannaberg (1894) put forward the hypothesis that they were produced by the conjugation of two asexual parasites within a red corpuscle, but the fact that cases of malaria with very numerous asexual parasites as a rule produce fewer crescents than those cases with few parasites, is strongly against this hypothesis. In heavy infections one often finds two parasites in one red cell, whereas in mild infections doubly infected red cells are exceedingly scarce, and yet many crescents may be produced. Some observers (Stephens and Christophers, 1908) suggest that they develop from special asexual spores.

(*b*) *It would appear that crescents develop from the asexual spores, due to a development of some immunity or resistance towards the latter.* Some cases of malaria produce gametes in large numbers, while others produce none at all. Their production appears to be determined by the appearance of conditions unfavourable to the asexual cycle. When the asexual spores find that their environment is becoming unsuitable, they undergo a transformation into a sexual generation, and thereby save themselves from destruction. In this new state they remain passive, awaiting their transference into a more suitable host.

If a special asexual spore were set aside to produce crescents, it would be expected that they would be produced in all cases independently of their environment.

The following eight points are in favour of this immunity hypothesis:—

1. Mild, chronic, and probably partially immune cases of malaria show, as a rule, a crescent-producing power fully ten times greater than the very acute cases. There are, however, a few exceptions to this, since acute cases sometimes produce a very large number of crescents.

2. It would appear that crescents are more likely to be produced in cases of long standing, where a certain amount of immunity has had time to develop.*

* Dr. James had a remarkable exception, in which a very large number of crescents appeared in a baby only fourteen days old (Case 2866).

3. Of cases with a history of previous attacks of malaria, 87 % developed crescents, whereas only 46 % of the cases with no history of previous attacks, produced crescents. It is reasonable to suppose that these cases, which had a history of previous attacks, were more immune than the primary cases.

4. Patients from 20 to 68 years of age produced more crescents than those below the age of twenty. This might be attributed to a greater power in adults of developing immunity as compared with the young, growing, patients. Many of the older patients, however, had been in the tropics for a long time, and had had previous attacks of fever. The young patients had not been long in malarial districts, and in most cases it was their first attack of malaria. The greater crescent producing power in older patients may, therefore, have been due to immunity developed from previous attacks.

5. A low percentage of haemoglobin is not so favourable for crescent production as a fairly high percentage. This again might be explained by the supposition that immunity to the asexual parasites is more likely to be successfully developed in cases where the blood standard is fairly healthy.

6. Cases with palpable spleens produced more crescents than those with impalpable spleens. According to Ross (1910) and Surveyor (1910), cases with enlarged spleen are more resistant than those without enlargement.

7. Greater numbers of crescents appeared to be produced in cases where the leucocyte count was high, which may again point to increased immunity.

8. Insufficient dosage of quinine would seem to favour the output of crescents. This may be due to the fact that small doses of quinine abate the disease, and so render the condition more favourable for the development of immunity, or it may be caused by quinine rendering the conditions less suitable for the asexual life of the parasite, so that it is induced to take on the sexual phase. It is probably correct to say that small doses of quinine, such as are given prophylactically, tend to produce crescent carriers, without curing the disease.

(*c*) *The crescents develop somewhere in the internal organs, and when completely developed, they appear suddenly in the peripheral blood.* Undeveloped crescents practically never appear in

the peripheral circulation. Here again, however, there was one remarkable exception in Dr. James' case (2866), in which all stages of crescents were found in the peripheral blood. (Plate V, figs. 7-14.)

(*d*) *A period of ten days elapses between the appearance in the peripheral blood of the asexual spores, and the crescents which are produced from these spores.* In other words, the crescents take ten days to reach maturity in the inner organs before they appear in the peripheral blood. As a general rule, the crescents appear in the peripheral blood on the fifth day after the attack of fever, and increase in number for four or five days more, so that they are most numerous on about the tenth day after the height of the fever. Those crescents appearing on the fifth day after the paroxysm, correspond to asexual parasites existing in the blood five days previous to this paroxysm. Asexual parasites can exist in the blood in numbers as great as 2,000 per c.mm., and more rarely 10,000 per c.mm. without producing any temperature reaction. They gradually increase in number by sporulation till they are numerous enough to cause a paroxysm of fever. The numbers may then fall spontaneously or by quinine treatment, so that only a single paroxysm results. It is by the study of such single paroxysms that the time required for the appearance in the peripheral blood of the corresponding crescents can be best determined. In such cases, the graphs representing the numbers of asexual parasites and crescents show a striking similarity, the points in the crescent graphs occurring on the tenth day after the corresponding points in the graphs of the asexual parasites. See Charts in previous paper, Thomson (1911).

One case was extremely interesting, as the patient had nine successive daily paroxysms of fever, four paroxysms in the four days before admission, and five paroxysms on the next five days. Corresponding to these nine fever paroxysms or sporulations, there were nine outbursts of crescents into the peripheral circulation, each occurring on the tenth day after the corresponding fever paroxysm. The asexual parasites were rapidly destroyed by quinine after the ninth paroxysm of fever, and a corresponding diminution in the production of crescents is evident ten days later.

Another case, Thomson (1912), showed a tertian outburst of

crescents into the peripheral circulation following upon tertian asexual sporulations. The first outburst of crescents occurred five days after admission, and would correspond to a fever paroxysm occurring five days before admission. The patient had had fever for several days before admission. On the tenth day after the administration of quinine, the crescents showed a more or less decided fall in number.

(*e*) *There is evidence to show that the duration of life of a crescent cannot be more than twenty days.* During the first ten days of this period they are developing somewhere, but not in the peripheral blood. They then appear in the peripheral blood. The majority of them perish in the peripheral blood in a very few days. This must be so, for if they lived for say four days or longer, then the summit of the crescent curve could not be a sharp point as it always is, especially when the numbers are great. In certain cases the number of crescents remains high for some days, the graph resembling a kind of plateau containing several sharp peaks. See Charts, Thomson (1911). The explanation of this plateau and apparent persistence of crescents is simple, for if no quinine is given the asexual parasites remain alive, even though there is no fever to indicate their presence, and help in producing new crescents. The source of crescents is not cut off, so that the supply is replenished by new broods of crescents, appearing every day or on alternate days or irregularly, according as the fever or asexual sporulation occurs every day, on alternate days, or irregularly.

The sharp peaks on the plateau show that the crescents are dying rapidly, but that their numbers are replenished by fresh broods coming into the circulation.

In no case is there a plateau formed when quinine has been given in doses of thirty grains daily ten days previous to the height of the crescent curve. If a plateau be formed, and quinine is then administered in large doses, its effect will not be manifested for about ten days, because although it very quickly reduces the source of supply, yet those crescents which commenced to develop during the previous ten days are not affected, but continue to appear in the peripheral blood, replenishing the loss. Hence, though quinine makes the crescents disappear from the peripheral blood more quickly than they would otherwise do without its administration,

yet its effect is not due to any direct destructive action in the crescents themselves. It is due indirectly to the destruction of the asexual spores from which the crescents are developed. The length of time that crescents will remain in the peripheral blood, therefore, depends upon the persistence of the asexual parasites. If immunity develops so strongly that the asexual parasites disappear, or if they are destroyed by quinine, then the crescents also will disappear in due course.* In cases where immunity remains, but is only sufficient to keep the number of asexual parasites in check, crescents may continue almost indefinitely. Crescents have been observed to continue in the peripheral blood for eight weeks (Surveyor, 1910). Many observers have noticed this long continuance of crescents, which has led to the fallacious belief that the individual crescents persist for a long time in the peripheral blood. The charts already referred to, show that the majority of crescents must die in the peripheral blood in a very few days. The sharpness of the peaks in the crescent graphs shows that the majority of them must perish in the peripheral blood in one or two days. Observations in many cases of malaria with crescents, 50 or more, show that crescents may be reduced to numbers below the detectable limit in the peripheral blood in less than 18 days by the administration of 20 to 30 grains of quinine daily. This dosage of quinine reduces the number of asexual parasites to below the detectable limit in five days, so that the source of supply is cut off in five days. Those crescents produced from the asexual parasites on the fifth day of quinine will appear in the peripheral blood ten days later, that is, on the 15th day after giving quinine. Three days more have to be accounted for. This may either be the duration of life of a crescent in the peripheral blood, or it may represent crescents developed from surviving asexual parasites after five days' administration of quinine.

In placing the duration of life of a crescent at 20 days, the greatest maximum is, therefore, given, viz., ten days' growth in the inner organs and ten days' life in the peripheral blood. It

* Cases are frequently met with in which the crescents quickly disappear spontaneously without quinine treatment. This is probably due either to the spontaneous disappearance of the asexual parasites or due to the fact that the asexual parasites cease to form crescents on account of conditions being favourable for the development of the former. In other words, crescents may disappear spontaneously due to a very high or a very low immunity to the asexual parasites.

would probably be more accurate to place this space of life as ten days in the inner organs and one to three days in the peripheral blood.

The graphs of the asexual parasite resemble very closely indeed the graphs of the crescents, the latter occurring ten days later than the former. The rise in both curves is rapid, and the fall in both is at first rapid, then as the numbers become fewer, the fall tails away very gradually till none are left. This alone is sufficient to show that the lifetime of a crescent after its ten days of growth to maturity is very short, for since quinine rapidly destroys the asexual source, and not the crescents themselves, then the crescents would necessarily accumulate, and show a death-rate curve entirely different from that of their producers.

These, then, are the conclusions regarding the source, growth and life of crescents deduced from enumerative observations on the peripheral blood alone. These observations were made by means of a special pipette, Thomson (1911), which enables one to estimate with considerable accuracy and quickness, the number of parasites per c.mm. contained in the peripheral blood; and it should be noted that the most valuable information thus obtained was got, not from single daily observations, but from enumerations made several times daily over a continuous period of some weeks.

(*f*) There is no evidence sufficient or definite enough to support the hypothesis that crescents are able by a process of parthenogenesis to revert back into the asexual phase. The forms described by Schaudinn as parthenogenetic were most probably either atypical sporulation forms due to quinine (Pl. V, figs. 42-45), or else they were partially developed crescents which had found their way into the peripheral circulation (figs. 10 and 11). These spherical, partially developed crescents are probably also the form of parasite described by Craig (1906) as conjugation forms.

(*g*) In all our observations we have found no evidence that male crescents flagellate while in the circulating peripheral blood, thereby fertilising the female crescents, as propounded by Mary Rowley-Lawson (1911).

(*h*) We have found no evidence, therefore, that relapses in malaria are caused by parthenogenesis of crescents, but believe that relapses are entirely due to resistant forms of the asexual phase

which have escaped the action of quinine (Ross and Thomson, 1910). This subject has already been thoroughly discussed, James (1913). He believes with Bignami (1912) that the asexual parasites may develop resistance to quinine just as trypanosomes do towards the arsenic preparations, and that these resistant forms by reproducing bring about the relapse.

III. DEDUCTIONS MADE FROM AUTOPSY SMEARS OF THE INNER ORGANS

The total number of cases in which autopsy smears were examined was 194. The general analysis of these cases was as follows:—

	CASES.
Parasites on admission, and parasites at autopsy	33
Parasites on admission, and pigment only at autopsy ...	10
Parasites on admission, and negative at autopsy	11
No record of blood examination on admission, and parasites at autopsy	5
No record of blood examination on admission, and pigment at autopsy	3
No parasites in blood on admission, and parasites at autopsy	18
No parasites in blood on admission, and pigment at autopsy	31
Negative for malaria on admission, and negative at autopsy	83
Total	194

Obviously the only cases from which we were able to draw any definite conclusions regarding the development of crescents were those 33 cases (all malignant tertian) which showed parasites in the peripheral blood on admission, and parasites in the autopsy smears. Information regarding the persistence of crescents during quinine treatment was obtained from some of the other cases. In order to analyse the first 33 cases properly, it was necessary for us to be able to recognise the various stages of crescents from the youngest to the adult forms. I believe that we managed this successfully, and we have depicted them in Plate V (figs. 7-14) and (figs. 22-30). The various stages in the development of the asexual parasites are shown (figs. 1-6), and (figs. 16-21). It will be noticed that the initial stages of the crescent before it takes on its characteristic

shape, resemble to some extent the initial stages of the asexual parasite. To distinguish between them, the staining should be as perfect and as deep as possible. The best results were obtained by staining with Hasting's or Wright's stain in the usual way, and then, further staining for 24 hours with Giemsa, and finally clearing away any stain deposit by rinsing the slide rapidly in orange-G.-Tannin. Good results can be obtained, however, by the ordinary method of Giemsa staining. In all its stages, the crescent differs from the asexual parasite in the staining of the protoplasm. The protoplasm of the latter stains a definite blue, whereas the crescent in all its stages stains a greyish blue or steel grey, with sometimes a yellowish tinge. The colour is also fainter than in the asexual parasite, a very good contrast is seen in fig. 6, in which is depicted the two forms of the parasite lying side by side. This happy coincidence was found in the peripheral blood of the remarkable case, 2866, already mentioned (p. 88). There are, however, further differences. The chromatin of the crescent stains more faintly than that of the asexual parasite, and appears to be made up of small granules, and it never segments into separate masses as occurs in the segmenting forms of the latter. Probably the greatest distinguishing feature between the asexual parasite and the crescent, however, is the distribution of the pigment. In the former, the pigment is practically always in one dense definite mass, at first placed eccentrically, later becoming more central. In all stages of the crescent the pigment is always definitely scattered, except in the adult form of the female crescent, where it is more or less concentrated. Finally, another important distinguishing feature is the shape. Even at a very early stage the crescent may take on the typical crescent shape so characteristic of the adult forms; more often, however, the young forms are round or oval, and when about three-quarters grown they become more oval, and then elongate, finally becoming crescentic. The youngest form of the crescent may be distinguished from the youngest asexual form, from the fact that it is heavily pigmented for its size, whereas no pigment is found in the youngest asexual parasite.

Most of these observations on the morphology of the developing crescent were made in the remarkable case, 2866,—a negro baby, age three weeks, which died shortly after admission to Ancon

Hospital, Panama. It had had fever during fourteen days previous to admission, according to the mother's statement. Numerous anophelines were found in the house, and it had, probably, been infected soon after birth. This case is a remarkable one, because it exhibited in the peripheral blood, not only all stages of the asexual form of the malignant tertian parasite, but, in addition, all stages of the developing gametes or crescents. It is not very common to find adult asexual forms of the malignant tertian parasite in the peripheral blood, and it is certainly most exceptional to find developing crescents. The next problem was that of determining the time required for the development of the crescent from the youngest to the adult form. It was already deduced from peripheral blood observations that the period of development was ten days in the inner organs. Among the 33 cases containing parasites at autopsy, seventeen cases showed crescents at some stage of development, and by a careful analysis of these we were able to find confirmation of the above deduction. On the assumption that the crescents develop from the youngest form of the asexual parasite, and further assuming that large doses of quinine quickly kill off the asexual, but not the sexual forms, then a case treated for ten days with quinine should show only adult crescents in autopsy smears. Cases treated for five days should show only half grown to adult forms of the crescent. Cases treated for about three days should show gametes from about one quarter-grown upwards, and untreated cases should show the asexual forms from which the gametes develop, and at the same time all stages of developing gametes or crescents. By carefully noting the amount of quinine treatment given before death and the various stages of crescents found at autopsy, we were able to obtain strong confirmatory evidence that our assumptions were correct. The details of these seventeen cases were as follows:—

Four cases were untreated with quinine, having died almost immediately after admission to hospital.

1. Case 2653. Autopsy smears of the spleen and bone marrow showed many sporulating asexual parasites, and a few very young developing crescents, but no adult forms.

2. Case 2866. The spleen and marrow smears showed all stages of the asexual cycle and all stages of crescents.

3. Case C. S., treated by Christian Scientist. The spleen and marrow showed very numerous sporulating asexual parasites, and a few crescents from the very youngest to half-grown forms.

4. Case C. The spleen and marrow showed numerous young asexual parasites and all stages of developing crescents as well as adult forms.

Three cases died after one day's treatment with quinine.

5. Case 2873. Received 65 grains of quinine. Autopsy smears showed scanty segmenting asexual forms, and a few crescents in all stages of development.

6. Case 2. Received 100 grains of quinine, and showed numerous asexual parasites and crescents of all stages at autopsy.

7. Case 2707. Received 30 grains quinine, and showed all stages of asexual and sexual parasites at autopsy.

Three cases died after two days' quinine treatment.

8. Case 1. Received 60 grains of quinine per day, total 120 grains. The spleen and marrow showed many young asexual forms and very scanty crescents from the youngest upwards.

9. Case 2. Received 120 grains of quinine in 26 hours, all forms of asexual parasites and all stages of crescents except the very youngest were found at autopsy.

10. Case 3. Received 100 grains of quinine in 26 hours. The autopsy showed all stages of asexual parasites and all stages of crescents but the youngest.

One case died after three days' quinine treatment.

11. This case had 125 grains of quinine during these three days. Autopsy showed all stages of asexual parasites, and also young crescents up to half-grown forms. It should be noted, however, that the young crescents were accompanied by an asexual generation in full development.

One case died after four days' quinine treatment.

12. This case received 45 grains of quinine daily for four days. The autopsy showed degenerating half-grown asexual parasites, and only half-grown to adult crescents.

Two patients died after five days of quinine treatment, 30 grains daily.

13. Case 1. The autopsy showed very scanty asexual parasites and numerous crescents from half-grown to adult forms.

14. Case 2. Autopsy showed no asexual parasites, and only half-grown to adult crescents.

The remaining three cases 15, 16 and 17 died after 10 days' quinine treatment of an intercurrent disease.

15. Autopsy showed no asexual parasites, and only three-quarters to adult crescents. (Quinine treatment 45 grains daily).

16. Autopsy showed no asexual parasites, and only one crescent (adult) was found. (Quinine treatment 45 grains daily).

17. Autopsy showed no asexual parasites, and only three-quarters to adult crescents. (Quinine treatment 15 grains daily).

From these cases, it would seem to us that the evidence goes to support the deduction that quinine cuts off the asexual source of supply of crescents, but that it does not destroy the crescents themselves at any stage of development, and that it would appear that the period required for their full development in the spleen and marrow is about ten days.

IV. FURTHER REMARKS

Some further observations regarding the persistence of crescents and pigment were brought forth in this research. In two cases adult crescents were found in autopsy smears after ten and eleven days' treatment with quinine (45 grains daily). And in seven cases malarial pigment was found in the spleen and marrow smears after 10, 11, 12, 13, 21, 24, 32 and 47 days' treatment with quinine (30 grains daily). In a few cases showing parasites on admission, no pigment was found in the autopsy smears. These as a rule had a long course of quinine (45 grains daily) for 25 to 40 days.

It was observed by Dr. James that some cases of blackwater fever showed very little evidence of pigment in the autopsy smears, even although numerous parasites had been present in the peripheral blood before the attack of blackwater, and only a few days before death. It is difficult to account for this. While working on the cultivation of malaria in vitro, it was noted by Dr. J. G. Thomson and myself that the malarial pigment persists in the culture tubes for months, and, in fact, it would seem to persist in the tubes indefinitely.

Regarding the problem of parthenogenesis, we have found no

evidence that this occurs in crescents, and it is quite probable that the forms described by the few who claim to have seen parthenogenesis, were either developing gametes, or else they were atypical sporulating schizonts, vide James (1913). In anaemic patients, and where a considerable amount of quinine has been given, the sporulation is often atypical (figs. 42-45). These forms are, in fact, unhealthy or degenerating parasites. The staining of the protoplasm is very faint, and the number of spores produced is much less than the normal number. Forms exactly similar are seen in cultures after four or five days. Sometimes in the same culture tube, the asexual parasite may pass through two, three, and even four generations, but the latter generations show signs of decay in that the number of spores produced is much less, only 6 to 10 being developed instead of the usual 24 to 32. The staining of the protoplasm also became very faint, and often it takes on a faint pinkish colour instead of the usual blue. The more advanced degenerated sporulation forms show only a mass of granular pinkish chromatin.

Finally, we have found no evidence in autopsy smears of the resting stages of the malarial parasite described by Craig, and it has been pointed out by James (1913), that these were probably developing gametes, such as depicted in figs. 10, 11, 27 and 28. In a few autopsy spleen smears we found bodies as depicted in figs. 46, 47 and 48. These were rather scarce, and we have been unable to determine what they may be.

V. SOME OBSERVATIONS ON THE FLAGELLATION OF THE MICROGAMETOCYTES

In Panama, flagellation occurred as early as four minutes after the blood was drawn. In Liverpool, the crescents became spherical in about four minutes, but they seldom flagellated until about twenty minutes after the withdrawal of the blood.

The time of flagellation varies in the same blood, some may flagellate in twenty minutes, some after fifty minutes. Whether the time depends on the temperature or not, I am unable to state. Sir Ronald Ross has been able to observe flagellation occurring even when the slide was kept on ice. I am inclined to believe that the cause of flagellation is osmosis. The crescents become spherical

by the passage of fluid into them, and this in turn gives the necessary stimulus for the flagellation to occur. It is generally admitted that breathing on the drawn blood is a good practice if one wishes to get successful flagellation, and it is even better still to add a little water to the blood. Most probably this addition of moisture or water makes osmosis more rapid. In normal blood in the body the osmotic pressure within and without the corpuscles must be about equal, but when the blood is withdrawn, coagulation occurs in about four minutes, and certain lime salts are deposited. The osmotic pressure in the coagulating blood fluid must, therefore, undergo a change, and a flow will take place into the corpuscle and into the body of the crescent, causing it to become spherical. I carried out a few experiments with the intention of bringing support to this hypothesis, and found that if a hypertonic saline solution were added to the drawn blood, the crescents did not become spherical, and they did not flagellate. When hypertonic saline solutions are added to blood, the blood corpuscles tend to shrink and become distorted, due most likely to a flow of fluid from the inside of the corpuscle into the more powerfully-concentrated fluid without. It has been observed by J. G. Thomson and myself that crescents do not become spherical in malaria cultures made after Bass's method. This is probably due to the same cause. In the cultures the blood is defibrinated, and glucose is added, but whether the persistence of the crescent shape is due to the defibrination, or due to the addition of glucose, I am unable to state. In a previous paper I pointed out that flagellation of crescents occurs in the blood from patients who have been subjected to a long and continuous quinine treatment, which shows that quinine does not readily destroy the vitality of crescents. When crescents are studied in wet films under the coverslip, it is noticed that polar bodies, usually two, appear on the margins of the spherical forms, later, if the crescent be a male, exceedingly active and struggling movements can be seen within the body of the parasite, and later, the flagella are extruded. These lash about with great activity and rapidity, and finally get free from the spherical body. Often they do not manage to get free, and they can be observed in rapid movement for a long time, even an hour or more, when the movement gradually gets more sluggish and they

apparently die. In stained preparations it would appear that a considerable amount of chromatin is extruded (figs. 31-36), and it is highly probable that this extruded chromatin forms the polar bodies observed in the wet film. In figs. 31 to 34 are depicted what appears to me to be female crescents which have become spherical. They show extruded chromatin, and also a circular pinkish area within the protoplasm. This circular pinkish area contains a centrally-placed dark chromatin dot.

In contrast to these, the male crescents which have flagellated (figs. 35, 36, 40 and 41) show a shrunken body, with extruded chromatin and also several flagella, which appear to arise each from a dot of chromatin within the body of the parasite. Figs. 38 and 39 show male spherical crescents which have not yet flagellated. The latter figure shows what appears to be the coiled-up flagella within the body of the crescent. The flagellum is often extruded in the form of a loop, and later, one end of the loop gets free; sometimes two flagella lie together as one thick flagellum. The number of flagella set free by each microgametocyte is a disputed point. Mary Rowley Lawson (1911) seemed to believe that only one flagellum came from each, but I think that there can be little doubt that the usual number is from four to eight, and fig. 41 would appear to show that even ten can be extruded from one parasite. Whether the number varies or whether it is always the same, I am unable to state. Mary Rowley Lawson also believed that flagellation and fertilisation in the gametocytes occurred in the circulating blood of the human patients. I have been unable to find any supporting evidence of this hypothesis. Spherical crescents are never seen in stained preparations of the circulating blood, provided the smears are dried quickly. I have never had the good fortune to see a free flagellum penetrate into the macrogametocyte. Fig. 37 represents a free flagellum found in a stained moist-chamber-preparation.

Before concluding, I wish to thank Dr. W. M. James for his most valuable assistance, and for his kindness in placing all his material at my disposal. My gratitude is also due to Dr. Henry Carter, and the American Medical Staff in general on the Panama Canal Zone.

VI. CONCLUSIONS AND SUMMARY

Deductions from Peripheral Blood Observations on Crescents

1. Crescents are produced from ordinary asexual spores of *P. falciparum*, due to a development of immunity towards the latter.

2. They develop somewhere in the internal organs, and then appear suddenly in the peripheral blood.

3. The period required for their development is about ten days.

4. Crescents do not generally live more than a few days in the peripheral blood.

5. Crescents may be present in the peripheral blood during periods as long as eight weeks, not because the individual crescents survive for that time, but because their numbers are constantly replenished from surviving asexual forms.

6. Fresh broods of crescents come into the circulating blood daily, or every other day, or irregularly, according as the asexual sporulations occurring ten days before, were quotidian, tertian, or irregular.

7. Quinine has no direct destructive action on crescents, either during their development or afterwards, but it destroys the asexual source of supply.

8. Quinine in doses of 20 grains daily reduces the crescents to numbers less than one per cubic millimetre of blood within three weeks.

Deductions made from Autopsy Smears of the Inner Organs

9. The crescents take about ten days to develop into the adult state in the inner organs.

10. They develop chiefly in the bone marrow and in the spleen.

11. The protoplasm of the crescent in all stages of development stains a faint greyish blue often with a yellow tinge, in contrast to the deeper blue of the asexual schizonts.

The chromatin of the crescents stains fainter, is less abundant and more finely granular than that of the schizonts, and it never breaks up into masses as occurs in the segmenting schizonts.

The pigment in the developing crescents appears early even in the youngest forms, and it remains scattered until the adult stage is reached, whereas in the schizont the pigment in all stages is more or less concentrated into one mass.

The shape of the developing microgametocyte is often spherical. Three-quarters grown forms may even be spherical. Sometimes, however, crescentic and oval-shaped forms may be found very early in development.

12. Adult crescents have been found in autopsy smears of the spleen and bone marrow, after eleven days' quinine treatment (45 grains daily).

13. Malarial pigment has been found in spleen and marrow smears after 47 days' quinine treatment (30 grains daily).

14. No evidence was obtained of parthenogenesis in crescents.

Observations on the Flagellation, &c.

15. Crescents become spherical in about four minutes after the blood is drawn, which appears to coincide with the time of coagulation.

16. Crescents may flagellate in four minutes after the blood is drawn, but some may not flagellate till after fifty minutes.

17. The crescents in patients subjected to thorough and continuous quinine treatment are able to flagellate.

18. The spherulation of crescents is probably due to osmosis, which in turn probably stimulates the actual flagellation.

19. The polar bodies seen in crescents which have become spherical are extruded chromatin.

20. The microgametocytes are capable of extruding 4, 6, 8 or 10 flagella (microgametes).

21. There is no reason to believe that crescents ever flagellate in the circulating blood of a patient.

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EXPLANATION OF PLATE V

All figures were drawn from stained preparations.
Magnification, 1,500 diameters.

Figs. 1 to 6. Show various stages of the malignant tertian asexual parasite found in the peripheral blood of the case 2866.

Fig. 6. Shows a segmenting schizont on the left and a three-quarters grown crescent on the right. These two were found lying together as painted.

Figs. 7 to 14. From the peripheral blood of case 2866, represent the various stages in the development of the gamete or crescent.

Figs. 15 to 21. From spleen smears of case 2866, show all stages in the development of the asexual phase of the malignant tertian parasite. Fig. 15 represents a large spleen cell which has ingested four young parasites.

Figs. 22 to 30. From spleen smears of case 2866, show successive stages in the development of the gamete or crescent.

Figs. 31 to 34. From moist-chamber-preparations, made by Dr. James, represent female crescents which have become spherical (macrogametocytes).

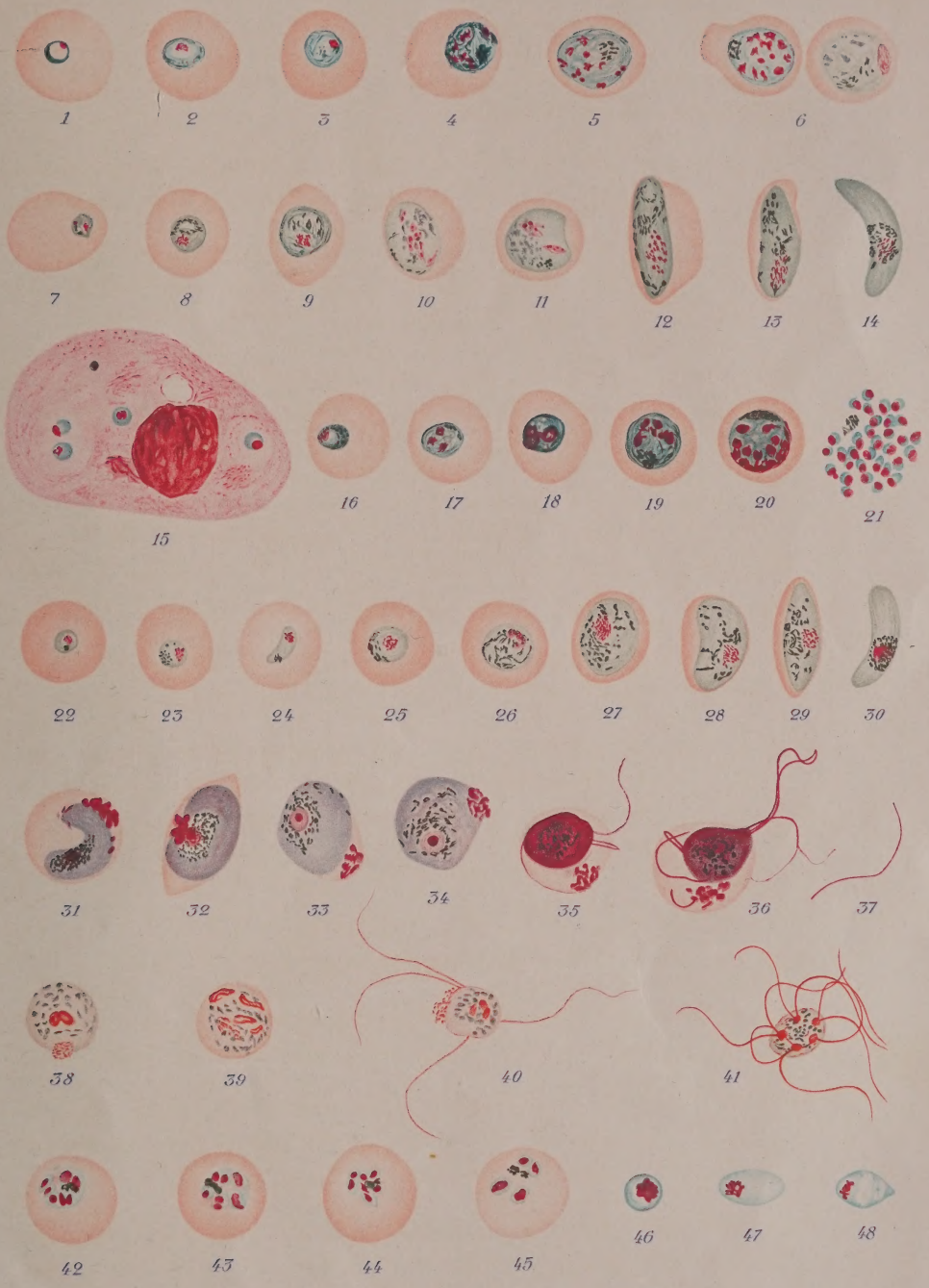
Figs. 35, 36, 40 and 41, represent flagellating crescents (microgametocytes).

Fig. 37. Shows a free flagellum (microgamete).

Figs. 38 and 39. Represent microgametocytes which have not yet flagellated. Fig. 39 shows the coiled-up flagella.

Figs. 42 to 45. From a smear of the placenta, show atypical sporulation of the malignant tertian parasite due to the action of quinine administration.

Figs. 46 to 48. From autopsy spleen smears, case 3295, represent bodies of undetermined nature. They do not appear to be malarial in origin.



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